Please replace the paragraph beginning at **page 10, line 28**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The percent sequence identity between a "variant" Iss polypeptide and a full-length Iss polypeptide, *e.g.*, SEQ ID NO:2, is generally determined by aligning the residues of the two amino acid sequences (i.e., a candidate amino acid sequence and the amino acid sequence of SEQ ID NO:2) to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. A candidate amino acid sequence is the amino acid sequence being compared to an amino acid sequence present in SEQ ID NO:2. A candidate amino acid sequence can be isolated from an avian *E. coli*, or can be produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, two amino acid sequences are compared using the Blastp program, version 2.1.2, of the BLAST 2 search algorithm, as described by Tatusova, et al. (*FEMS Microbiol Lett* 1999, 174:247-250), and available from the worldwide web at ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, percent identity is referred to as "identities."

Please replace the paragraph beginning at **page 12, line 17**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

An isolated "variant" nucleic acid sequence of the present invention is a nucleic acid sequence that has at least 87%, preferably at least about 90%, and most preferably at least about

95%, but less than 100%, nucleic acid sequence identity or homology to a the nucleotide sequence of the corresponding wild type nucleic acid molecule, e.g., a DNA sequence comprising SEQ ID NO:22. However, a variant nucleic acid molecule of the invention may include nucleotide bases not present in the corresponding wild type nucleic acid molecule, as well as internal deletions relative to the corresponding wild type nucleic acid molecule. As used herein a nucleic acid "subunit" is a biologically active portion or region of a full-length *iss* nucleic acid sequence, *e.g.*, SEQ ID NO:22, or a portion or region of an *iss* nucleic acid sequence that encodes a biologically active subunit of an Iss polypeptide. The percent identity between a "variant" nucleic acid sequence of the present invention and a wild type nucleic acid molecule, e.g., SEQ ID NO:22, is determined by aligning the residues of the two polynucleotides (i.e., the candidate nucleotide sequence and the nucleotide sequence of the coding region of SEQ ID NO:22) to optimize the number of identical nucleotides along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of shared nucleotides, although the nucleotides in each sequence must nonetheless remain in their proper order. A candidate nucleotide sequence is the nucleotide sequence being compared to SEQ ID NO:22. A candidate nucleotide sequence can be isolated from an *E. coli*, preferably an *E. coli* obtained from a bird, or can be produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, two nucleotide sequences are compared using the Blastn program, version 2.1.2, of the BLAST 2 search algorithm, as described by Tatusova, et al. (*FEMS Microbiol Lett* 1999, 174:247-250), and available from the worldwide web at ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including reward for match = 1, penalty for mismatch = -2, open gap penalty = 5, extension gap penalty = 2, gap x_dropoff = 50, expect = 10, wordsize = 11, and filter on. In the comparison of two nucleotide sequences using the BLAST search algorithm, percent identity.

Please replace the paragraph beginning at **page 21, line 3**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

When the avian *E. coli iss* sequence was examined with GENE INSPECTOR software (Textco, Inc., West Lebanon, New Hampshire), it was found that the avian *E. coli* Iss protein is predicted to have an isoelectric point of approximately 8.47, and at pH 7, is expected to have a net charge of +2.05. An Iss polypeptide is predicted to be approximately a 10-11 kD protein containing 102 amino acids that is resistant to acid hydrolysis. Additionally, based on the Iss polypeptide's predicted folding characteristics and hydropathy plots, Iss is likely to have a number of accessible sites, for example, sites not buried in the bacterial membrane, that are antigenic.

Please replace the paragraph beginning at **page 35, line 3**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The Merrifield synthesis method commences from the carboxy-terminal end of the peptide using an alpha-amino protected amino acid. Fluorenylmethyloxy-carbonyl (Fmoc) or t-butyloxycarbonyl (Boc) protective groups can be used for all amino groups even though other protective groups are suitable, and the first protected amino acids can be esterified to chloromethylated polystyrene resin supports. The polystyrene resin support is preferably a copolymer of styrene with about 0.5 to 2% divinyl benzene as a cross-linking agent which causes the polystyrene polymer to be insoluble in certain organic solvents. See Carpino *et al.*, J. Org. Chem., 37:3404 (1972); Meinenhofer, Int. J. Peat. Pro. Res., 11:246 (1978); and Merrifield, J. Am. Chem. Soc., 85:2149 (1963). The immobilized peptide is then N-deprotected and other amino acids having protected amino groups are added in a stepwise manner to the immobilized peptide. At the end of the procedure, the final peptide is cleaved from the resin, and any

remaining protecting groups are removed by treatment under acidic conditions, for example, with a mixture of hydrobromic acid and trifluoroacetic acid. Alternatively, the cleavage from the resin may be effected under basic conditions, for example, with triethylamine, where the protecting groups are then removed under acidic conditions. The cleaved peptide is isolated and purified by means well known in the art, for example, by lyophilization followed by either exclusion or partition chromatography on polysaccharide gel media such as SEPHADEX G-25, or countercurrent distribution. The composition of the final polypeptide may be confirmed by amino acid analysis after degradation of the polypeptide by standard means.

Please replace the paragraph beginning at **page 35, line 27**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The synthesis may use manual techniques or be completely automated. For example, an APPLIED BIOSYSTEMS 431A PEPTIDE SYNTHESIZER (Foster City, Calif.) or a BIOSEARCH SAM II automatic peptide synthesizer (Biosearch, Inc., San Rafael, Calif.) can be employed following the directions provided in the instruction manual and reagents supplied by the manufacturer. Disulfide bonds between Cys residues can be introduced by mild oxidation of the linear peptide by KCN as taught in U.S. Pat. No. 4,757,048 at column 20.

Please replace the paragraph beginning at **page 41, line 24**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Responding mice are given a final booster consisting of about 5-100 μg, preferably 25-50 μg of antigen, preferably without adjuvant, administered intravenously. Three to five days after final boosting, spleens and sera are harvested from all responding mice, and sera is retained for use in later screening procedures. Spleen cells are harvested by perfusion of the spleen with a

syringe. Spleen cells are collected, washed, counted and the viability determined via a viability assay. Spleen and SP2/0 myeloma cells (ATCC, 10801 University Boulevard, Manassas, VA 20110-2209) that have been screened for HAT sensitivity and absence of bacterial contamination are combined, the suspension pelleted by centrifugation, and the cells fused using polyethylene glycol solution. The "fused" cells are resuspended in HT medium (RPMI supplemented with 20 % fetal bovine serum (FBS), 100 units of penicillin per ml, 0.1 mg of streptomycin per ml, 100 μM hypoxanthine, 16 μM thymidine, 50 μM 2-mercaptoethanol and 30 % myeloma-conditioned medium) and distributed into the wells of microtiter plates. Following overnight incubation at 37°C in 5% CO_2, HAT selection medium (HT plus 4 μM aminopterin) is added to each well and the cells fed according to accepted procedures known in the art. In approximately 10 days, medium from wells containing visible cell growth are screened for specific antibody production by ELISA. Only wells containing hybridomas making antibody with specificity to Iss, or GST-Iss, are retained. The ELISA is performed as described above, except that the primary antibody added is contained in the hybridoma supernatants. Appropriate controls are included in each step.

Please replace the paragraph beginning at **page 44, line 22,** with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Examples of adjuvants or carriers that may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL + TDM + CWS) in an emulsion containing 2% squalene/TWEEN 80 (tradename) emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of

B8 antibodies directed against an immunogenic polypeptide containing an Iss antigenic sequence resulting from administration of the polypeptide (or a nucleic acid encoding the polypeptide) in immunogenic compositions or vaccines that are also comprised of the various adjuvants.

Please replace the paragraph beginning at **page 56, line 27**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

B9 Polypeptides prepared from the pGEX-6P-3 expression vector yielded a glutathione S-transferase-Iss ("GST-Iss") fusion polypeptide product that is readily purified from the bacterial lysates by affinity chromatography under mild, non-denaturing conditions. Specifically, a bacterial sonicate is applied to a column of GLUTATHIONE SEPHAROSE 4B (Pharmacia Biotech Inc., Piscataway, N.J.) at 4° C and washed three times with 10 bed volumes of 1X PBS. Glutathione elution buffer (10 mM reduced glutathione in 50 mM Tris-HCl (pH 8.0)) is added to the column and incubated at room temperature (about 22-25° C) for 10 minutes, and the fusion protein is eluted. Eluates recovered from the column contain the fusion protein.

Please replace the paragraph beginning at **page 57, line 7**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

B10 Alternatively, after expression of GST-Iss in *E. coli*, the bacteria are lysed by sonication, and the insoluble material is pelleted and removed, and the supernatant passed through a slurry of GLUTATHIONE SEPHAROSE 4B (Pharmacia Biotech Inc., Piscataway, N.J.) to permit binding of the GST-Iss fusion polypeptide to the Sepharose beads. To remove GST-Iss from other cellular proteins, the "bead-bound" fusion polypeptide is pelleted by centrifugation and washed with 1X PBS. The desired product is eluted from the Sepharose by the addition of reduced glutathione (Pharmacia Biotech Inc., Piscataway, NJ). Upon removal from the

SEPHAROSE beads, the purified GST-Iss fusion product is cleaved into Iss and GST by a site-specific protease, such as PRECISSION PROTEASE (Pharmacia Biotech Inc., Piscataway, NJ), and the remaining GST is separated from Iss by the same procedure used to purify the GST-Iss fusion polypeptide. The resulting polypeptide products are then analyzed by SDS-PAGE.

B10

Please replace the paragraph beginning at **page 60, line 8**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Restriction enzyme digest plasmid fragments and amplification products were separated by horizontal gel electrophoresis. Amplified fragments were identified by size, excised from the agarose and purified using GENECLEAN™ (Bio101, La Jolla, Calif.) or the WIZARD PCR CLEAN-UP SYSTEM (Promega, Madison, WI). The identities of the amplicons were further confirmed by sequencing according to the procedures described below. To prepare probes, isolated fragments were labeled using a non-radioactive, random-primed DNA labeling kit (GENIUS I Labeling and Detection Kit, Boehringer Mannheim, Indianapolis, Ind.).

B11

Please replace the paragraph beginning at **page 61, line 1**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The first series of cycles were the labeling reactions, wherein an IRD40-dATP was added to the extended primer sequence. The second series of cycles was the termination reaction for which 4 µl of the labeling reaction was added to 2 µl of each ddNTP termination mix before cycling. Unincorporated label was removed from the products by ethanol precipitation according to manufacturer's directions. Samples were separated on 4.0 % LONG RANGER acrylamide gels (FMC, Rockland, ME) and analyzed via a LI-COR 4000 LR automated sequencer.

B12

Please replace the paragraph beginning at **page 61, line 18**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

B13

The prepared *E. coli* DNA was added to a master mix consisting of 47.5 - 53.5 µl ddH_20, 10.0 µl of 10X PCR Buffer II (Perkin Elmer or Promega, Madison, WI.), 0.5 µl of AMPLITAQ DNA polymerase (Perkin Elmer), 1.0 µl of 0.1 mM of each appropriate primer and 4.0 µl or 8.0 µl of 25 mM $MgCl_2$.

Please replace the paragraph beginning at **page 61, line 22**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

B14

The amplification cycles were as previously described except that the annealing temperature was 49 °C or 51.8 °C depending on the primer pair used. Amplified fragments for ligation into the expression vector pGEX-6P-3, were digested with *Bam*HI and *Eco*RI to produce sticky ends for the ligation process. Amplified fragments for ligation into pGEM-T vector have "A" overhangs left by Taq polymerase. Restriction enzyme digest plasmid fragments and amplification products were separated by horizontal gel electrophoresis. Amplified fragments were identified by size, excised from the agarose and purified using the WIZARD PCR CLEAN-UP SYSTEM (Promega, Madison, WI). T7 DNA ligase was used to ligate amplification fragments into the vectors. The identities of the amplicons were further confirmed by sequencing according to the procedures described below.

Please replace the paragraph beginning at **page 67, line 22**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

To prepare samples for analysis by flow cytometry, bacteria are grown in Brain Heart Infusion (BHI) broth for 18 hours at 37° C. Bacteria are then washed and resuspended in buffer. Monoclonal antibodies specific for Iss are added to each bacterial suspension and allowed to incubate for 30 minutes. Suspensions are pelleted, washed thoroughly, and incubated with FITC-labeled, goat anti-mouse Ig conjugated antibody for 30 minutes at 0° C. After incubation, suspensions are pelleted, washed thoroughly, fixed with paraformaldehyde, and analyzed by flow cytometry using a FALSCALIBUR (Becton Dickinson, San Jose, CA) (Otten *et al.*, Flow Cytometry Analysis Using the Becton Dickinson FACScan, John Wiley & Sons, New York, pp. 5.4.1-5.4.19 (1992)).

Please replace the paragraph beginning at **page 68, line 8**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Cloning of *iss*. The primers for amplification of *iss* were designed using the PRIMERSELECT program of LASERGENE (DNAStar, Madison, WI) based on the DNA sequence in Horne et al. (*Avian Dis.*, 44: 179-184 (2000); GenBank Acc. No. AF042279; SEQ ID NO:1). To facilitate cloning, these primers were modified to add restriction sites (Bam*HI* on the upper primer and Eco*RI* on the lower primer at the 5' ends). The modified nucleotides are present in the primers as non-italicized text, and the italicized nucleotides hybridize with nucleotides present in SEQ ID NO:1 or the complement thereto. The primers were *iss* pVAX1 Upper, 5'AGTGGGGATCC*TAACAATGCAGGATAATAAGATGA* (SEQ ID NO:23); and *iss* pVAX1 Lower, 5'ATGCGGAATTC*TGTAGGGAGCCCAGAAGTA* (SEQ ID NO:24).

Please replace the paragraph beginning at **page 68, line 19**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Based on the unmodified primers, amplification conditions recommended by PRIMERSELECT were determined, and these were used to amplify *iss* from the virulent avian *E. coli*. DNA template from the *E. coli* isolate used in the amplification was prepared as follows. A colony was lysed in a solution containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 50 µg/ml proteinase K by incubation at 55°C for 10 minutes followed by incubation at 80°C for 10 minutes. After dilution with a 2x volume of ddH_2O, cellular debris were removed by a brief centrifugation (30 seconds at 10,000 x g), and the supernatant containing the DNA was transferred to a fresh tube. Five microliters of the DNA sample was added to a 45 µl reaction volume of Master Mix in a 200 µl thin walled microfuge tube. Master Mix was 27 µl of ddH_2O, 5 µl of 0.1 mM of the primer *iss* pVAX1 Upper, 0.5 µl of 0.1 mM of the primer *iss* pVAX1 Lower, 0.25 AMPLITAQ (5 Units/ml)(Ampligene Kit, Perkin-Elmer, Foster City, CA), and 4 µl of 25 mM $MgCl_2$. A Perkin-Elmer PE2400 thermocycler was used to amplify *iss*. The amplification protocol used was 95°C for 5 minutes; 9 cycles of 95°C for 1 minute, 51.6°C for 30 seconds, and 72°C for 30 seconds; 25 cycles of 94°C for 30 seconds, 51.6°C for 30 seconds, and 72°C for 30 seconds; 72°C for 7 minutes; 4°C soak.

Please replace the paragraph beginning at **page 69, line 7**, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Using this procedure, a 400 base pair amplicon was generated. This amplicon was gel purified and removed from the low melt agarose using WIZARD PCR CLEAN-UP KIT (Promega, Madison, WI). The *iss* amplicon was digested with Eco*RI* and Bam*HI* to prepare for cloning. At the same time, pVAX1, a plasmid specifically designed for use in DNA vaccines